The mitochondrial aspartate–glutamate carrier (AGC) imports glutamate with a proton into mitochondria and exports aspartate [1]. The transporter domain shares common characteristics with other members of the mitochondrial carrier family [2], but it has an extra N-terminal domain with EF-hand calcium-binding motifs [3].

The aspartate–glutamate carrier is involved in linking ubiquitous and tissue-specific metabolic pathways that are separated by the mitochondrial inner membrane. In human, there are two human isoforms, aralar1 (*SLC25A12*) and citrin (*SLC25A13*) [3]. Both isoforms operate in the malate–aspartate shuttle, which plays a key role in mammalian cells by transporting NADH reducing equivalents from the cytosol to mitochondria. In addition, the operation of the malate–aspartate shuttle is important to supply mitochondrial aspartate for liver-specific processes catalysed by Citrin, namely the urea cycle and gluconeogenesis, and for brain-specific processes catalysed by Aralar1, such as myelination in the central nervous system [4]. Mutations in the *aralar1* and *citrin* genes cause global cerebral hypomyelination and type-II citrullinaemia, respectively [4].

The functional and structural analysis of the aspartate–glutamate carrier may enable us to address the molecular basis of calcium-regulated and proton-coupled import of glutamate in exchange for aspartate. Further, it will also allow us to put the observed mutations into context and help to explain how they affect the overall function of the aspartate–glutamate carrier.

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4P14

Identification and characterization of a new Na^+/H^+ antiporter in *Neisseria meningitidis*

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Neisseria meningitidis (Nm) is the causative agent for meningitis, affecting the population worldwide. Recently, the Na^+ transport has been associated with the virulence of some pathogenic bacteria [1]. It is therefore important to understand the mechanisms underlying the Na^+ cycle across the membranes of this pathogenic bacterium. Na^+/H^+ antiporters, one type of the transporters playing a role in Na^+ transport, are known to be important in sodium and pH homeostasis. A search for Na^+/H^+ antiporters was performed in *N. meningitidis* genome, and two hits were retrieved, showing significant similarity to antiporters of the NhaC and NhaE groups. The NmNhaE was expressed in *Escherichia coli* KNabc, a strain in which Na^+/H^+ antiporters are absent, and thoroughly investigated.

Upon induction, the plasmid-coded NmNhaE antiporter enabled *E. coli* KNabc to grow in the presence of 500 mM NaCl and 200 mM LiCl from pH 6.5 to 8.5. Membrane vesicles from *E. coli* KNabc cells containing over-produced NmNhaE were subjected to ion transport studies. The optimal pH for Na^+-transport was 7.0, with a binding constant of 0.29 mM and a maximal activity of 38% of dequenching, and 0.85 mM and 29% of dequenching for Li^+ transport. The higher affinity to sodium suggests that NhaE plays an important role in sodium and pH homeostasis in *N. meningitidis*. An amino acid sequence comparison between the NmNhaE and the sequences of several known bacterial Na^+/H^+ antiporter families showed that this antiporter affiliates in the NhaE group.

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4P15

Electrophysiological characterization of *Af*-Amt1 on a solid supported membrane

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The transport of ammonium/ammonia across biological membranes is mediated by a family of ubiquitous integral membrane proteins, the ammonium transport (Amt) proteins. Although high-resolution crystal structures of four Amt proteins are currently available [1-5], the substrate identity and transport mechanism are still controversially discussed [6]. Current functional data using two-electrode voltage clamp experiments of protein expressed in *Xenopus* oocytes [7,8], measuring the radioactive uptake of methyl ammonium, an alternative substrate in whole cells [9] or following pH variation with fluorescent probes in proteoliposomes [1], yielded variable and discrepant results. To conclude on the substrate identity (NH_4^+ or NH_3) and the transport mechanism we are studying Amt-1 from *Archaeglobus fulgidus* adsorbed on a solid supported membrane (SSM) sensor. Such methodologies have been recently used to study the details of the downhill sugar/H^+ symport of the lactose permease LacY from *Escherichia coli* [10,11], or the pH regulation of the Na/H^+ antiporter NhaA from *E. coli* [12]. If Amt proteins are electrogenic transport proteins, a charged substrate transport into the proteoliposomes will generate transient currents, allowing an *in vitro* characterization of this class of membrane proteins.

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4P16

Nitric oxide regulation of heart mitochondrial bioenergetics and calcium handling

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Nitric oxide (NO) is a free radical produced within the heart by NO synthases (NOS) located in different compartments. The localization of mitochondrial NOS within the organelles in charge of energy metabolism allows for a tight control of respiration, ATP and superoxide anion production, apoptosis, mitochondrial biogenesis, mitoK$_{ATP}$ activation and MPT formation. In turn, Ca^{2+} plays a central role in energy supply and demand matching by transmitting changes in excitation–contraction coupling to mitochondria. The uptake of Ca^{2+} is accomplished by the mitochondrial Ca^{2+} uniporter (MCU) and the extrusion by the mitochondrial Na^{+}/Ca^{2+} exchanger. Ca^{2+} activates NADH production in the Krebs cycle and triggers MPT in cell death. Heart mtNOS activity was measured in permeabilized myocytes and in energized mitochondria and was shown to depend on mitochondrial Ca^{2+} concentration. In this study, the effects of NO on Ca^{2+} influx were characterized by exposing isolated guinea pig heart mitochondria to physiological and pathological concentrations of NO released from two NO donors: GSNO and SNAP. Effective NO concentrations released by the donors were assessed by EPR. Mitochondrial NO production, $\Delta\Psi$ and Ca^{2+} uptake were followed simultaneously in a fluorometer (Quantamaster, Photon Technologies International) using DAF-FM, TMRE, Calcium Green and fura-FF as probes. Isolated mitochondria O_2 consumption was assessed using a XF96 analyzer (Seahorse Bioscience). State 4 O_2 uptake was not modified by 500 μM GSNO or SNAP. The addition of Ca^{2+} to the respiration medium produced a 20% enhancement in state 4 O_2 consumption and this effect was abolished by GSNO or SNAP. Control energized mitochondria were submitted to 10 μM Ca^{2+} pulses up to a final concentration of 80–100 μM (200–450 nmol/mg protein), showing no significant alterations in matrix volume and $\Delta\Psi$. In the presence of GSNO or SNAP (25 to 100 μM), Ca^{2+} uptake was slower and extramitochondrial Ca^{2+} concentration increased. When single 50 μM Ca^{2+} pulses were added, mitochondria treated with NO donors for 2 min showed a decreased (40–50%) accumulation rate of Ca^{2+} with an IC_{50} of 400 μM (180 nM NO). The addition of l-arginine or NOS inhibitors (L-NAME and nNOS inhibitor I) to control mitochondria produced slight changes in Ca^{2+} uptake and in DAF-FM signal. These results suggest that Ca^{2+} and NO may act as signals that coordinate changes in cytosolic workload and mitochondrial energy metabolism in the heart.

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